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THE ANTIBODIES FOR SHEEP BLOOD AND COMPLEMENT OF THE AQUEOUS HUMOR IN NORMAL AND IMMUNIZED RABBITS

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The antibodies and complement of the aqueous humor in normal and immunized conditions have not been investigated to any considerable extent in systematic manner. As it is possible that a careful study of the aqueous humor from the immunologic point of view might throw light on such problems as the permeability of blood vessels for various immune bodies, the work now to be reported in brief was undertaken. The anterior chamber of the eye with its bloodless and transparent corneal covering would seem to be an ideal place to secure a suitable special fluid for repeated examination by immunologic methods. The few previous investigations of special interest in connection with this work will be considered in the discussion of my results.

METHODS

Healthy rabbits weighing from 1 to 2½ kilos were used. Blood was obtained in the usual manner from the ear vein under sterile precautions. Aqueous humor was obtained by means of a fine, sterile needly (No. 23) connected with a tube graduated in hundredths of c c; the humor was removed slowly, always without pressure or suction, and with special care to avoid any injury to the iris or other structures. The slightest suspicion that any admixture with blood or plasma had taken place as an immediate result of the puncture eliminated that particular fluid from the tests. The amount obtained in each case varied from 0.2 to 0.27 c c. Usually serum and aqueous humor were obtained in the morning, before the animals were fed, and tested the same day. In any case the serum and fluid were kept in the icebox always until used. To inactivate heating at 56 C. for 30 minutes was practiced. All dilutions were made with sterile 0.9% salt solution. Carefully washed sheep corpuscles were used in the hemolysis tests and fresh guinea-pig serum served as complement unless otherwise stated. Usually the hemolytic mixture consisted of 0.1 c c of serum or aqueous humor, either in full strength or diluted, 0.1 c c of a 5%

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suspension of corpuscles, and 0.003 c c of guinea-pig serum, the total quantity being made 0.3 c c by adding salt solution. After incubation for 2 hours the tubes were placed in the icebox and the results recorded the next morning. The hemolytic titer was determined by the highest active dilution of serum or humor which gave a definite trace of positive reaction under careful controls, and frequently several tests were made to determine the accuracy of the results. The tests for agglutinins for red corpuscles were made with heated (56 C.) serum or humor, the quantity being 0.1 c c or less, 5% suspension of well washed corpuscles 0.1 c c, and salt solution to make 0.3 c c, incubated for one hour, the final reading being made the next morning, the tubes in the meantime being kept in the icebox. The precipitin tests were made with fresh sheep serum diluted with salt solution, 0.25 c c of the dilution in each tube, 0.05 c c of the serum or humor being introduced at the bottom so as to secure a sharp line of contact; the results were determined after one hour at room temperature. In the opsonin tests, fresh as well as heated (56 C.) serum or humor was used; 0.1 c c of the serum or humor and of the corresponding dilutions was mixed with 0.1 c c washed sheep corpuscles (5% suspension) and 0.1 c c of pleural exudate, carefully washed and produced by injecting rabbits with aleuronat suspension; sometimes the cream of rabbit blood was used to furnish the leukocytes. The leukocytes were kept always at body temperature to exclude deterioration. The opsonic mixtures were incubated at 37 C. for one hour when smears were stained by Wright's method. From 50 to 150 leukocytes were studied in each case to determine the percentage of phagocytic cells. The opsonic titer was fixed by finding the highest dilution of serum or humor that exercised greater opsonic effect and induced more phagocytes than in the control mixture without serum or humor.

ANTIBODIES FOR SHEEP BLOOD IN SERUM AND AQUEOUS HUMOR IN NORMAL RABBITS

Two sets of 3 animals in each were studied and the results are illustrated in tables 1 and 2. The results show that there is a considerable variation in the lysin content of the serum of normal rabbits, the titer running from 96 to 768, and that the titer appears to increase somewhat after a first decrease with repeated bleedings, perhaps as the result of a stimulation of the bloodmaking organs. As to the aqueous humor, it is to be noted that on first puncture it seemed usually not to contain any lysin; occasionally a trace (titer of 3) could be detected.

TABLE 1

TITERS OF HEMOLYTIC AMBOCEPTOR IN SERUM AND AQUEOUS HUMOR OF NORMAL RABBITS

Days Between Bleedings	Titer of Serum			Right or Left Eye	Days Between Punctures	Titer of Aqueous Humor		
	Rabbit 1	Rabbit 2	Rabbit 3			Rabbit 1	Rabbit 2	Rabbit 3
	192	96	768	L	...	0	0	0
2	192	48	768	R	...	0	0	0
2	192	96	384	L	4	0	0	0
2	96	96	192	R	4	0	0	0
2	96	96	192	L	4	3	3	3
3	192	192	384	L	3	3	3	3
5	192	192	384					

Serum heated at 56 C. for 30 minutes; aqueous humor not heated; complement, guinea-pig serum, 0.003 c c.

TABLE 2

TITERS OF LYTIC AMBOCEPTOR, AGGLUTININ AND OPSONIN FOR SHEEP CORPUSCLES IN SERUM AND AQUEOUS HUMOR OF NORMAL RABBITS

Days Between Bleedings and Punctures of Anterior Chambers	Rabbit 1					Rabbit 2					Rabbit 3				
	Serum			Aqueous Humor		Serum			Aqueous Humor		Serum			Aqueous Humor	
	Lysin	Agglutinin	Opsonin	Lysin	Opsonin	Lysin	Agglutinin	Opsonin	Lysin	Opsonin	Lysin	Agglutinin	Opsonin	Lysin	Opsonin
	192	10	....	0	..	96	20	....	0	..	384	20	....	0	..
7	192	10	640	0	20	48	10	320	0	..	384	10	80	3	..
6	96	10	$\frac{640}{5}$	0	$\frac{10}{0}$	48	10	320	3	5	192	20	320	3	5
5	192	10	$\frac{1280}{10}$	0	$\frac{10}{0}$	96	20	640	0	5	1536	40	640	3	10
4	192	10	$\frac{2560}{10}$	0	$\frac{20}{0}$	96	10	640	0	5	1536	20	640	0	10
3	192	10	$\frac{2560}{10}$	0	$\frac{20}{0}$	96	10	320	0	10	1536	20	320	3	10
2	192	10	....	0	$\frac{40}{0}$	96	10	—	0	10	1536	20	....	3	10
1	384	10	$\frac{640}{5}$	0	$\frac{40}{0}$	768	10	1280	0	20	384	20	1280	24	20

Serum and aqueous humor heated to 56 C. for lysin and agglutinin tests, but not for opsonin tests. Figures under the line under opsinin, Rabbit 1, given opsonin titer of heated serum and humor. No agglutinin for sheep corpuscles in aqueous humor. No trace of precipitin for sheep serum in either serum or humor. Both chambers punctured at same time.

The relative content of lytic amboceptor in aqueous humor and blood stands in an approximate ratio of about 1 to 256. With repeated puncture at intervals of over two days a small amount of lytic amboceptor occasionally appears in the humor. In the course of the experiments it was observed that after removal of the aqueous humor the content of the anterior chamber may return to the normal condition usually within 24 hours. In some special experiments punctures of the anterior chamber were made at intervals of 30 minutes and, as shown in table 3, the amount of lysin in the fluid withdrawn on the second puncture was vastly greater than that in the fluid of the first, the titers in the native humors running from 24 to 96 or about one-fourth to one-eighth the strength of the corresponding serum. This result shows how easily the blood vessels permit the passage of lysin under conditions of changed pressure without any inflammatory or other changes. It is noteworthy that, as already indicated, in about 24 hours the normal conditions of the aqueous humor are restored fully as a rule.

TABLE 3

Content of Hemolytic Amboceptor for Sheep Corpuscles in Aqueous Humor on Two Punctures, 30 Min. Apart

Punctures	Rabbit 1	Rabbit 2	Rabbit 3	Remarks
First.............	3	3	3	Aqueous humor heated at 56 C. for 30 minutes; complement guinea-pig serum, 0.003 c c
30 minutes later	48	24	96	

The normal rabbit serum unheated causes lysis of sheep corpuscles in dilutions up to 1 : 24, but the aqueous humor in full strength, that is, one part of fluid to one part of 5% suspension of corpuscles, is without any lytic effect.

Next to be considered was the amount of complement in normal rabbit serum, whether any complement occurs in the normal aqueous humor, and whether complement enters the aqueous humor on puncture of the anterior chamber.

Using the serum of a rabbit that was found to contain an average amount of amboceptor titer 192, I tried to complement this amboceptor with the aqueous humor of several normal rabbits, but in no case did the humor obtained on the first puncture in the quantity of 1 c c serve to complement the amboceptor. One example is shown in table 4. On second puncture, 30 minutes later, however, the humor was found to contain some complement that clearly must have passed into the anterior chamber from the blood as a result of the changes in pressure. Compared with the amount of complement in the serum, the amount in the aqueous humor obtained on second puncture was on an average about one-fourth the concentration of the complement of the serum. Here I may point out that the amount of complement in normal rabbit serum varies greatly, being alwavs less than in guinea-pig serum, from ⅛ to ¼ as much. There is no parallelism between the amount of complement and amboceptor in the normal rabbit.

As shown in table 2, the agglutinin titer of normal rabbit serum for sheep corpuscles is between 10-20, occasionally being as high as 40; in the aqueous humor, however, no agglutinin was found in any case on the first puncture.

TABLE 4

REACTIVATION OF RABBIT SERUM HEATED TO 60 C. FOR 10 MINUTES WITH NORMAL RABBIT SERUM AND ORIGINAL AQUEOUS HUMOR AND AQUEOUS HUMOR DRAWN 30 MINUTES AFTER FIRST PUNCTURE

Rabbit Serum Heated at 60 C. for 10 Minutes, C c	5% Suspension of Sheep Corpuscles, C c	Fresh Rabbit or Guinea-Pig Serum or Aqueous Humor, C c	Hemolysis			
			Fresh Rabbit Aqueous Humor		Fresh Rabbit Serum	Fresh Guinea-Pig Serum
			First Puncture	Second Puncture		
0.1	0.1	0.1	0	+++	++++	++++
0.1	0.1	0.05	0	++	++++	++++
0.1	0.1	0.025	0	+	+++	++++
0.1	0.1	0.0125	0	0	+	+++
0.1	0.1	0.00625	0	0	±	++
0.1	0.1	0.003	0	0	0	+
—	0.1	0.1	0	0	0	0
0.1	0.1	—	0	0	0	0

Total amount made 0.3 c c by adding normal salt solution.

The titer of the opsonin of fresh normal rabbit serum for sheep corpuscles was found to run from 80-640, and that of normal aqueous humor of the same rabbits to vary from 5-20. After heating the serum, the titer was found to be about 5 and after heating the aqueous humor no opsonic effects could be demonstrated.

On repeated puncture of the anterior chamber, the titer of the thermolabile opsonin of the aqueous humor rose occasionally to 40, but it is to be emphasized that probably as the result of repeated bleedings during this period, the titer of the serum of the rabbits had increased to 2,560. While the thermostable opsonic substance in the normal serum had risen to a titer of 10, the aqueous humor still remained free from any demonstrable such opsonin. In no case was any trace of precipitin for sheep proteins found in either the serum or the aqueous humor of normal rabbits.

RELATIVE CONCENTRATION OF HEMOLYSIN AND OTHER ANTIBODIES IN SERUM AND AQUEOUS HUMOR OF RABBITS IMMUNIZED WITH SHEEP BLOOD

As stated, the titer is represented by the highest dilution of serum or humor that gave a definite trace of lysis. The animals received 30 c c of defibrinated sheep blood on one injection and were subsequently bled every 2 or 3 days, while the anterior chamber was punctured with the greatest care at intervals of at least 2 days. The lysin curves of the serum of the animals were similar in outline but varied in height, one rabbit giving a very high curve and 2 others moderately high curves. The 2 latter presented a negative phase for 2 days after the injection. In all cases the lysin production set in on the 4th day and rose steadily, reaching the highest point on the 10th day except in the animal that gave the highest curve; in this the apex was reached on the 14th day. In all the animals the descent of the curve began on the 14th day and reached the normal in one case on the 40th day, in another the titer was still a little higher than normal on the 51st, while the rabbit with the very high curve died on the 40th day without any other lesions except a progressive emaciation.

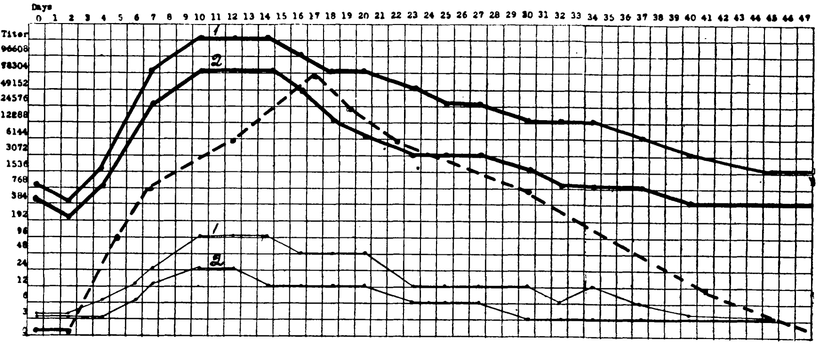
TABLE 5

HEMOLYTIC AMBOCEPTOR IN SERUM AND AQUEOUS HUMOR OF RABBITS INJECTED INTRAPERITONEALLY WITH 30 C C OF SHEEP'S BLOOD

Days After Injection	Rabbit 1		Rabbit 2*		Rabbit 3	
	Serum	Aqueous Humor	Serum	Aqueous Humor	Serum	Aqueous Humor
Before (2 days)	768	3	384	3	384	3
2	384	3	384	3	192	3
4	1,536	6	3,072	3	768	12
6	24,576	12	98,304	6	6,144	24
7	98,304	24	78,642	48	24,576	24
10	393,216	96	....	768	98,304	24
12	393,216	96	....	..	98,304	24
14	393,216	96	....	1536	98,304	12
16	196,608	48	....	..	49,152	12
18	98,304	48	....	..	12,288	12
20	98,304	48	786,432	24	6,144	12
23	49,153	12	398,216	12	3,072	6
25	24,576	12	196,608	12	3,072	6
27	24,576	12	98,304	12	3,072	6
30	12,288	12	24,576	12	1,536	3
32	12,288	6	12,288	6	768	3
34	12,288	12	12,288	12	768	3
37	6,144	6	6,144	6	768	3
40	3,072	6	3,072	6	384	3
45	1,536	3	Died	..	384	3
51	768	3	....	..	384	3

* This rabbit produced an enormous amount of amboceptor, the titer going into the millions at the height. In the meantime the animal became extremely weak and thin so that bleeding and punctures had to be omitted at times.

In the aqueous humor of these rabbits there was a definite rise in lysin, running parallel with the lysin in the blood serum, but very much lower and somewhat less regular. The increase began on the 4th day in 2 animals and on the 6th in 1, reached the high point on the 10th or 12th day when a gradual fall began, the normal level being reached on the 30th-40th day. As shown in table 5, the hemolysin titer of the humor reached from 24 to 96, and as high as 1,536 in an extraordinary case; the ratios of the titer of the aqueous humor of these animals to the titers of the serum at the highest points of curves were 1:4,096 in two of the animals and 1: 16,384 in the third. At the time when the titer of the humor began to rise, the titer of the serum was from 756-1,536. We see that the relation between the titer of the aqueous humor and blood serum now is not the same as in the normal animal.



Hemolysin curves of blood serum (heavy solid lines) and of aqueous humor (fine lines) of Rabbits 1 and 2 each injected intraperitoneally with 30 c c of sheep's blood and precipitin curve (broken line) of serum of rabbit injected with 40 c c of sheep's blood intraperitoneally; no precipitin in the aqueous humor of this rabbit

Both precipitin and agglutinin appeared in the serum on the fifth day after the injection of 40 c c of sheep's blood in another set of rabbits (table 6) and increased in amount steadily, the precipitin reaching its height on the 16th day and the agglutinin on the 12th; in both cases a gradual fall set in; on the 16th day and on about the 50th day all evidence of these antibodies had disappeared from the blood. The aqueous humor of the same rabbits failed to show any trace of precipitin or agglutinin. It would seem as if there is some hindrance to the passage of these antibodies into the anterior chamber, possibly because of the size of their molecules.

The intraperitoneal injection of 40 c c of defibrinated sheep blood into rabbits was followed by a rise in the opsonic power of the fresh serum for sheep corpuscles beginning about the 5th day and reaching its highest point on the 16th day; on the 22nd day a fall began, the normal level being reached again on about the 49th day. There was a parallel rise in the opsonic power of the fresh (unheated) aqueous humor, the relation of the titer to that of the serum at the height of the curve being 160: 20,480 or 1 : 128.

The hemolytic power of the fresh serum of rabbits injected with sheep's blood showed only a slight increase when tested without the addition of complement of other source, and the aqueous humor under these circumstances was without any lytic effect. It is clear from this result that while there is a marked increase in amboceptor after the injection of sheep's blood, there is no apparent change or increase in the native complement of the rabbit serum.

TABLE 6

OPSONIN, AGGLUTININ AND PRECIPITIN IN SERUM AND AQUEOUS HUMOR OF RABBITS INJECTED INTRAPERITONEALLY WITH 40 C C OF SHEEP'S BLOOD

Days After Injection	Opsonin				Agglutinin in Serum	Precipitin in Serum
	Serum		Aqueous Humor			
	Unheated	Heated	Unheated	Heated		
	320	5	20	0	5	0
2	—	—	—	—	5	0
5	640	..	20	0	20	80
8	1,280	—	40	0	40	640
12	4,120	160	80	0	80	5,120
16	20.480	640	160	0	80	81,920
19	20,480	640	160	0	40	20,480
22	20,480	160	160	0	40	5,120
26	10,240	320	80	0		
30	2,560	80	40	0	40	640
35	1,280	20	40	0	20	
42	640	10	20	0	10	10
49	320	5	20	0	5	0

No agglutinin or precipitin in aqueous humor.

It appears that in the course of immunization with sheep blood the only antibodies to appear anew in the aqueous humor are lysin and opsonin.

DISCUSSION

According to Gatti,[1] the aqueous humor in normal cattle does not contain any hemolysin, but in 5 of 13 dogs this humor was lytic for rabbit corpuscles, according to Hughes and Carlson.[2] Becht and Greer,[3] however, found no lysin in the aqueous humor of 10 normal dogs, and they say that even dogs injected with rabbit's blood do not show any lysin or agglutinin in the aqueous humor. According to my observation, the aqueous humor of normal rabbits sometimes may contain a trace of lysin for sheep corpuscles. Generally speaking, it would seem that depending on individual variation the hemolytic titer of the aqueous humor of normal rabbits runs from 0-3. This variation may be related to the normal hemolytic titer of the serum which I found may vary between 96 and 768, repeated bleedings tending to increase the titer. I was not able to demonstrate any hemagglutinin in the normal rabbit aqueous humor, and the same is also true of precipitin for sheep serum; opsonin for sheep corpuscles, however, was found to be present in small quantities.

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[1] Biochem. Centrlbl., 1905, 4, p. 678.

[2] Am. Jour. Physiol., 1908, 21, p. 245.

[3] Jour. Infect. Dis., 1910, 7, p. 131.

On active immunization with sheep blood, the aqueous humor, under otherwise normal conditions, may contain lytic amboceptor in noticeably greater amount than normally. In no case did any hemagglutinin or precipitin appear in the aqueous humor after the injection of sheep blood. These observations are in full harmony with the results obtained by Hektoen and Carlson,[4] who found that after the injection of dogs with goat blood new lysin appeared in the aqueous humor but no agglutinin. But Wessely [5] makes the general statement that agglutinin and hemolysin exist only in a minimal amount in the intra-ocular fluid of the most highly immunized animals.

Metchnikoff [6] and Mesnil [7] noted that alexin was absent from the aqueous humor, and Metchnikoff believed that this was due to the fact that there are no leukocytes in this fluid. In my work, complement was never found in the aqueous humor of normal rabbits. Roemer,[8] Falloise,[9] Sweet,[10] and Miyashita [11] report similar observations. It is of interest to note that as a rule the pericardial and cerebrospinal fluids do not contain complement.

After immunization with sheep blood, specific precipitin for sheep serum develops in large quantities in the serum, reaching in one of my animals a titer of 81,920, but in no case was any trace of precipitin demonstrable in the aqueous humor. Von Dungern [12] also found that the aqueous humor of animals injected with the plasma of Maja aquinado was free from precipitin while the serum contained large quantities, and would remain free unless the iris became injured in some way. Becht and Greer[3] also found no precipitin in the aqueous humor of a dog injected with a large amount of rabbit blood.

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[4] Ibid., 1, p. 319.

[5] Klin. Monatsbl. f. Augenheilk., 1902, 40, p. 267.

[6] Ann. de l'Inst. Pasteur, 1895, 9, p. 438.

[7] Ibid., 1896, 10, p. 369.

[8] Arch. f. Augenheilk., 1906, 54, p. 207.

[9] Bull. de l'Acad. Roy. Belg., 1905, 5.

[10] Zentralbl. f. Bakteriol., 1903, 33, p. 208.

[11] Klin. Monatsbl. f. Augenheilk., 1910, 48, p. 293.

[12] Die Antikorper, Jena, 1903, p. 113.

On immunization new opsonin may make its way into the aqueous humor in rabbits just as Hektoen and Carlson[4] found it to do in dogs injected with goat blood. As hemolytic amboceptor may exist in the aqueous humor of rabbits without the presence of any complement, the humor nevertheless containing opsonin, it would seem that it concerns different substances.

In the course of my experiments the anterior chamber was punctured frequently at intervals, beginning 2 days after the injection, up to several days in animals injected with sheep blood, hence sheep protein might probably enter the aqueous humor. If antibodies can be produced locally, one would expect especially that there would be an accumulation in the anterior chamber, but such did not seem to be the case as the curve of the lysin was exactly like that of the serum only much lower, and no hemagglutinin or precipitin seemed to arise locally. My results seem to support the conclusions of Hektoen[13] that antibodies may enter the aqueous humor from the blood and lymph and not as the result of any local production.

CONCLUSIONS

The serum of normal rabbits contains lysin and opsonin, also agglutinin in small quantities for sheep corpuscles, but no precipitin for sheep serum.

The aqueous humor of normal rabbits contains a small amount of opsonin and lysin for sheep corpuscles, but no agglutinin or complement and no precipitin for sheep serum.

On immunization with sheep blood the antibodies mentioned accumulate in the blood, but the aqueous humor gives an increase in lysin and opsonin only, remaining free from the other antibodies.

On removal of aqueous humor by puncture, the new fluid that forms has a far greater amount of antibodies in it than the original fluid. The normal conditions are regained in about 24 hours.

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[13] Jour. Infect. Dis., 1911, 9, p. 103.